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# Transplantation of the *Sc* Melanoma in Fishes

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(Plates I-V; Text-figures 1-4)

MELANOMAS have been described in representatives of all major groups of vertebrate animals, but successful transplantations of these black neoplasms have been reported only in mammals and amphibians. We now report the successful implantation and growth of a melanoma in fishes when the tumorous fragments are placed in the host's integumentary tissues between the scales. Previously, attempts to implant fish melanomas into the eye or peritoneum had failed but some success had been attained in an intermuscular site.

We (Marcus & Gordon, 1953) found that the genetic constitution of the host was of paramount importance in determining the success or failure of the growth of transplanted tumor tissue. This is in line with results obtained with other experimental animals. In the transfer of melanoma cells in fishes, autotransplantations were most successful. Transplantation to animals closely related to the host were successful less frequently. No success was attained when tumor fragments were placed in genetically unrelated fishes.

The transplantation of melanoma fragments into the transparent integumentary tissues between the scales of fishes has permitted us to observe the progressive transformations of the tumor cells in the host. The events were traced under a compound microscope by a method somewhat similar to the one developed by Algire (1943), who utilized a transparent window in his study of the growth of melanomas in mice.

It was hoped that continuous observations of individual tumor cells would give us the ontogenetic history of the melanocytes, the primary cell type of the definitive melanoma. While the evidence we have obtained is still incomplete, it appears that some of the melanocytes transform into macromelanophores. These large pigment cells have been determined by genetic experiments of Gordon (1951) to be the initiating elements for the development of melanomas in the members of certain genetic stocks of fishes.

## Material and Methods

The fish melanomas successfully transplanted had originated spontaneously from a series of matings between Montezuma swordtails (*Xiphophorus montezumae*) carrying the dominant *Sc* gene, and common swordtails (*Xiphophorus helleri*), recessive for the same gene. The *Sc* gene in the normal *montezumae* is expressed by the presence of large black pigment cells or macromelanophores on the caudal fin. In *montezumae* × *helleri* hybrids of the first generation, the hypertrophic growth of macromelanophores produced much larger pigmented areas. By backcrossing the black-spotted hybrids to *helleri* swordtails, fish were obtained in which the caudal areas were intensely pigmented by macromelanophores. Atypical pigment cell growth led to the development of definitive melanomas at the site of abnormal pigmentation in the tail area, (Pl. I, Fig. 1; Pl. V).

In a preliminary statement, Gordon & Nigrelli (1949) described the *Sc* melanoma in *montezumae* × *helleri* hybrids, in part, as follows: "The corium is completely replaced by proliferating macromelanophores, which vary in size, shape and amount of melanin present, both within the same tumor and among similar tumors in different fish. The melanin-bearing cells at the periphery of the growth appear to be larger, show more numerous dendritic

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processes and are more loosely arranged than those towards the center of the tumor mass. There is considerable infiltration and destruction of muscle, bone and cartilage by these melanin-bearing cells. Numerous macrophages are invariably present at the periphery of the growth, with an occasional one containing melanin. In some regions of the growth the epithelium is thin and broken, probably as a result of the expanded corial growth. However, the epithelium in the region of the fins is often appreciably thickened. The tumor is especially characterized by extensive development of capillaries and sinuses together with numerous lacunae throughout, but especially at the periphery of the growth."

Hybrid fish bearing melanomas were placed for a few minutes in an anaesthetic solution of 1:3,000 of Sandoz' MS-222 (Tricaine methanesulfonate) to which the disinfectant Merthiolate (Lilly), 1:10,000, was added. Pieces of the melanoma were cut from the donor and teased apart in sterile "amphibian" saline (0.68% NaCl). Tumor fragments (0.2 to 0.8 mm) were inserted into the host as follows: A scale was raised slightly and then the untrimmed tumor particle was inserted by means of a blunt glass needle into the deepest part of the scale pocket in a region free of macromelanphores, far anterior to the caudal peduncle. No visible hemorrhages occurred during or following the operation. Some unimplanted tumor fragments were preserved in fixing fluids.

The black tissue implants were visible, owing to the transparency of the epidermis and scales, and were observed and photographed or drawn with the aid of the high power of a compound microscope. After suitable intervals some host animals with transplanted tumors were fixed in Bouin's and selected areas were sectioned. The slides were stained either with Masson's variant of Mallory's trichrome stain or with haematoxylin and eosin.

In the experiments and observations reported here, the names of cells follow the nomenclature recommended at the Third Conference on the Biology of Normal and Atypical Pigment Cell Growth (Gordon, 1953, and Fitzpatrick & Lerner, 1953):

Melanoblast: an embryonic cell potentially capable of producing melanin.

Melanocyte: a mature melanin-producing and melanin-containing cell.

Macrophage: a cell containing phagocytized melanin.

Melanophore: a pigment effector cell in lower vertebrate animals.

## Types and Histories of Transplants

(Tables 1 and 2, Pls. I and II)

*Autotransplants.*—An *Sc* melanoma fragment was implanted into an anterior, non-macromelanophore-bearing area of the skin of the same animal. Fate: Six of nine transplants developed successfully. Some host animals with their actively growing implants were fixed after

Table 1. Transplants of Spotted Caudal (*Sc*) Melanoma

| Number | Donor | Host | Fate | Remarks |
|---|---|---|---|---|
| | | | 9 Autotransplants | |
| 3 | h42*Sc* | Same | Temporary | Resorbed in 2 to 6 weeks |
| 6 | h42*Sc* | Same | Permanent | Some fixed after 8 months, others after 24 |
| | | | 9 Homotransplants Series "A"* | |
| 6 | h42*Sc* | h42*Sc* | Temporary | Persistent up to 5 weeks |
| 3 | h42*Sc* | h42*Sc* | Permanent | One degenerated after 2 months, then grew again |
| | | | 21 Homotransplants Series "B"* | |
| 3 | h42*Sc* | h42*sc* | Temporary | Resorbed in 4 to 5 weeks |
| 4 | h42*Sc* | h42*sc* | Negative | |
| 5 | h50*Sc* | h50*sc* | Temporary | Resorbed in 3 to 6 weeks |
| 9 | h50*Sc* | h50*sc* | Negative | |
| | | | 18 Heterotransplants | |
| 7 | h50*Sc* | h42*Sc* | Negative | |
| 1 | h50*Sc* | h42*Sc* | Temporary | Resorbed after 2 weeks |
| 3 | h42*Sc* | 182 | Negative | Host = *X. helleri* albino |
| 4 | h50*Sc* | 287 | Negative | Host = Spotted-belly *X. helleri* × *X. maculatus* hybrid |
| 3 | h50*Sc* | 281 | Negative | Host = *X. maculatus* |

*In homotransplants "A" donors and hosts were dominant for the *Sc* gene for macromelanophores on the caudal fin. In series "B" the hosts were recessive for the *sc* gene and did not have macromelanophores.

Table 2. Fate of *Sc* Melanoma Transplants

| | Auto-transplants | Homotransplants* Series "A" | Homotransplants* Series "B" | Hetero-transplants | Totals |
|---|---|---|---|---|---|
| Number Implants | 9 | 9 | 21 | 18 | 57 |
| Successful "Takes" | 6 | 3 | 0 | 0 | 9 |
| Non-persisting Cell outgrowths | 3 | 6 | 8 | 1 | 18 |
| Failures (No activity) | 0 | 0 | 13 | 17 | 30 |

* In homotransplants "A" the donors and hosts were dominant for the *Sc* gene for macromelanophores on the caudal fin. In series "B" the hosts were recessive for the *sc* gene and did not have macromelanophores.

eight, others at 24 months. The rates of growth of four autotransplants are given in Table 3.

*Homotransplants.*—A fragment of an *Sc* melanoma was removed from one tumorous fish and implanted into two kinds of its siblings. In series "A" both donor and host carried the dominant *Sc* gene for *spotted caudal* pattern. In series "B" the host was recessive for this factor. Fate: In group "A," three of nine transplants persisted as long as the host lived, which was eight to 24 months. In group "B" none of the transplants was successful. In both groups several transplants grew for a short period, three to six weeks, and then regressed and disappeared.

*Heterotransplants.*—In this group, the fishes providing the tumor tissue differed more widely in their genetic constitutions from the host animals. Some transfers of the *Sc* melanomas were made from tumorous hybrids to members of the succeeding generations from brother to sister matings; others were made to members of the backcross generation. Some hosts represented different species. Fate: All but one of 18 heterotransplants failed to make any growth at all; one grew for two weeks, then disappeared.

### Microscopic Observations of Implants *in situ* (Pl. III)

Immediately after a successful implantation, the tumorous fragment becomes surrounded by a light gray halo of free melanin granules. Within five to 36 hours, the free melanin granules are phagocytized by macrophages; they appear as black clumps. Within about one week most melanophages migrate to the edge of the scale and disappear. Eventually the area of the implant is cleared of pigment cell debris.

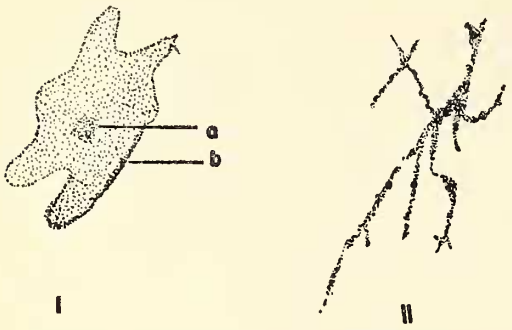
On the third to sixth day, dendritic processes of pigment cells emerge from the margin of the implant. Some of these are long and slender with pointed or clubbed tips, others are broad and sheet-like with short, spinous extensions.

After the sixth day, two kinds of melanocytes emerge from the implant. The smaller type (Text-fig. 1; Pl. IV, Fig. 1) has a few, thin pseudopodial processes, containing irregularly scattered melanin granules; it resembles the "small melanoblast" described by Grand & Cameron (1948) from tissue-cultures of fish melanomas. The larger type of melanocyte (Text-fig. 1; Pl. IV, Figs. 2 & 3) has a wide cell body, broad pseudopodial processes, and contains evenly distributed melanin granules; it resembles the cell that Grand & Cameron (1948) called a "large melanoblast." We believe it is a juvenile macromelanophore because we have observed the successive steps in the maturation of a similar cell (Text-fig. 2; Pl. IV, Figs. 3 & 4). When first seen, the young macromelanophore was elongate, broad, with-

Table 3. Growth of Seven *Sc* Melanoma Implants during Twenty-four Months

Size in Millimeters of Implants by Months

| Initial | 2 | 4 | 6 | 8 | 24 |
|---|---|---|---|---|---|
| | | Autotransplants | | | |
| 0.6 | 1.4 | 1.9 | 2.3 | 2.4 | Fixed |
| 0.4 | 1.7 | 2.0 | 2.1 | 2.2 | Fixed |
| 0.5 | 2.0 | 3.0 | 3.9 | 4.1 | 4.6 |
| 0.2 | 0.5 | 0.6 | 0.8 | 1.0 | Fixed |
| | | Homotransplants | | | |
| 0.7 | 2.5 | 3.6 | 4.2 | 5.1 | 5.3 |
| 0.7 | 1.9 | 3.2 | 3.3 | 3.3 | Fixed |
| 0.3 | 0.5 | 0.7 | 1.2 | 1.2 | 1.4 |




TEXT-FIG. 1. Diagrammatic representation of a large (I) and a small (II) melanocyte (cf. Pl. IV, Figs. 1 to 4). The large melanocyte is characterized by the accumulation of pigment at the center of the cell (a) and at the periphery (b). The small melanocyte is highly dendritic and appears darker. (About 50×.)

out pseudopodial processes, and contained evenly distributed melanin granules. After four days the cell was more discoid and had definite pseudopodial processes. The processes contained evenly distributed melanin granules, while the center of the cell had an increased amount of melanin. After six days the large melanocyte looked like a typical melanophore.

An apparent reversal was also observed; that is, a melanophore had changed into a melanocyte. When first seen the melanophore had many short, slender, melanin-containing pseudopodial processes. Four days later, the melanin granules were more evenly distributed throughout the cell. Its slender pseudopodial processes disappeared; it was now rounder and its processes quite short and broad. Six days later, its cell outline was still more rounded and then it had the characteristics of a typical melanocyte. Between the fourth and sixth day it had moved a distance of 30 micra.

The melanocytes possess considerable migratory activity; the melanophores do not. Text-figure 3 shows three melanocytes and a record of their movements: one cell (b) moved away from the implant while the other two cells (a, c) moved parallel to its margin. Usually, the cells became sedentary after three to six days.

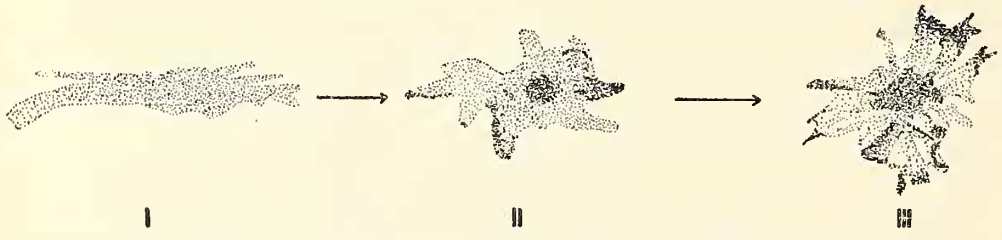
In order to distinguish the true pigment cells (that is, melanocytes and melanophores) from macrophages with their phagocytized melanin (melanophages), a small drop of a 1:1,000 solution of trypan blue in "amphibian" saline (0.68% NaCl) was placed under a slightly raised scale in a lightly pigmented portion of the skin. The dye filled the scale pocket and formed a diffuse blue area immediately beneath the scale. Within twenty-four hours, the particles of dye began to come together in a manner similar to the clumping of melanin granules around the freshly implanted melanoma fragment. Clumps of trypan blue particles, apparently within macrophages, passed to the edge of the scale and disappeared. No melanocytes, typified by their dendritic processes, picked up the color, nor did mature melanophores.

### HISTOLOGY OF TRANSPLANTS IN HOST TISSUE (Pl. IV, Figs. 5-7)

Cross-sections through the transplants revealed black tissues in the dermis so densely pigmented by hypertrophied macromelanophores that no cellular structures were definable, a condition that typifies melanosis. Only along the margin of the transplant were pigment cell processes seen. There was no obvious vascular response in the host's tissues that were in contact with the implant.

The invasive pigment cells spread along the ventral surfaces of the scales and along the external muscle fascia below the dermis. The pigment cells eventually invaded the central portions of the dermis of the host, and occasionally occupied the entire dermal area. The subcutaneous muscles of the host were rarely and only slightly involved.

The histology of the transplant differed from




TEXT-FIG. 2. Transformation of a large melanocyte into a macromelanophore as traced by study of a single cell, located at the edge of a growing autotransplant, and drawn at 48-hour intervals. When first seen (I), the cell had the characteristics of a large melanocyte. Later (II), it developed pseudopodial processes, attained more melanin pigment and rounded up somewhat (cf. Text-fig. 1). Still later (III), the same cell attained the morphological characteristics of a melanophore. (From camera lucida drawings, about 50×.)

that of the melanoma fragment originally inserted. The high vascularity, blood-filled lacunae and the many unpigmented or slightly pigmented melanocytes characteristic of the *Sc* melanoma were wanting in the persistent transplant. After eight or even after 24 months of activity, the implant assumed the histological characteristics of a melanosis, a condition produced by the hypertrophic growth of macromelanophores. This kind of abnormal growth of large pigment cells is found in spotted xiphophorin fish hybrids prior to the development of melanoma.

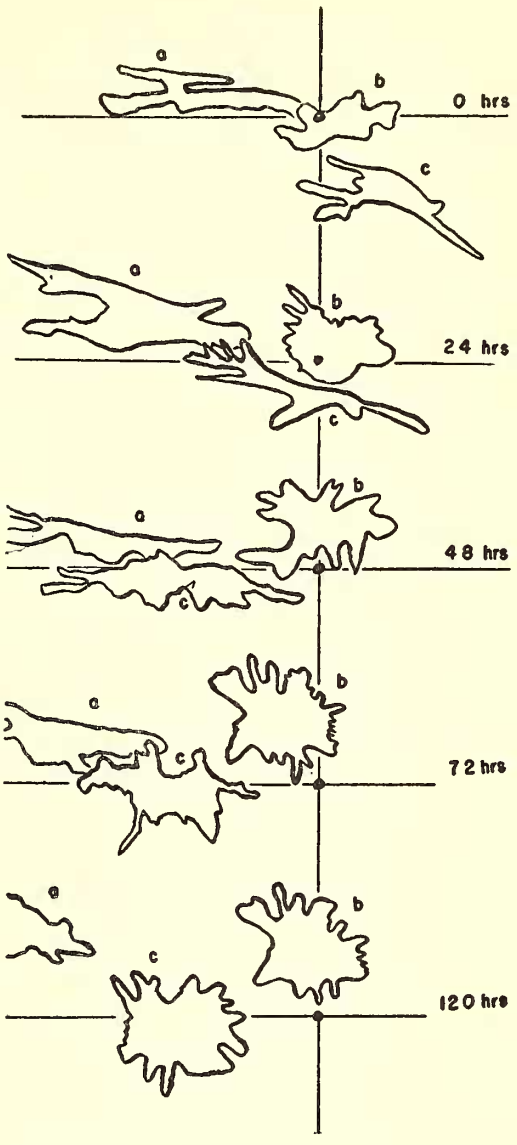
## Discussion

Gordon & Smith (1938), Grand, Gordon & Cameron (1941) and Levine (1948) have drawn attention to the morphological similarity between melanoma cells of xiphophorin fish and mammals. There are also physiological similarities between mammalian and piscine melanomas. For example, Algire & Legallais (1948) called attention to the minimal vascular reaction around the growth of implanted melanomas in mice. Similarly, there is no apparent vascular reaction in the skin of the fish after implantation of a melanoma fragment.

The growth characteristics of the transplantable melanotic tumors in axolotls, as described by Sheremetieva-Brunst & Brunst (1948) resemble those of fishes. In the early stages the invading cells of one amphibian tumor travel along paths established by connective tissues. From the corium, the melanophores break through the subcutaneous fascial tissue and penetrate the musculature. Not all melanotic tumors have the ability to penetrate the subcutaneous tissues, but they occupy most of the corial tissues.

With regard to the influence of genetic correspondence between the donor and host tissues for successful growth of transplantable melanomas, the situation in mice is revealing. The Harding-Passey mouse melanoma, according to Algire & Legallais (1948), may be transplanted to mice of many strains, but the Cloudman S-91 melanoma is successful only in members of the *dba* strain. In amphibia, Krontovsky (1916) reported that not even autotransplants of melanomas were successful. Those studied by the Brunsts were successful when introduced into axolotls of apparently different strains. Their success, however, may have resulted from better transplantation techniques.

Melanomas from hybrid fishes failed to grow when transplanted into the peritoneums or eyes of other strains, but some success was obtained with a tumor transfer into the superficial muscles of an albino swordtail (Pl. II, Fig. 2). In contrast, even with the better technique, no success

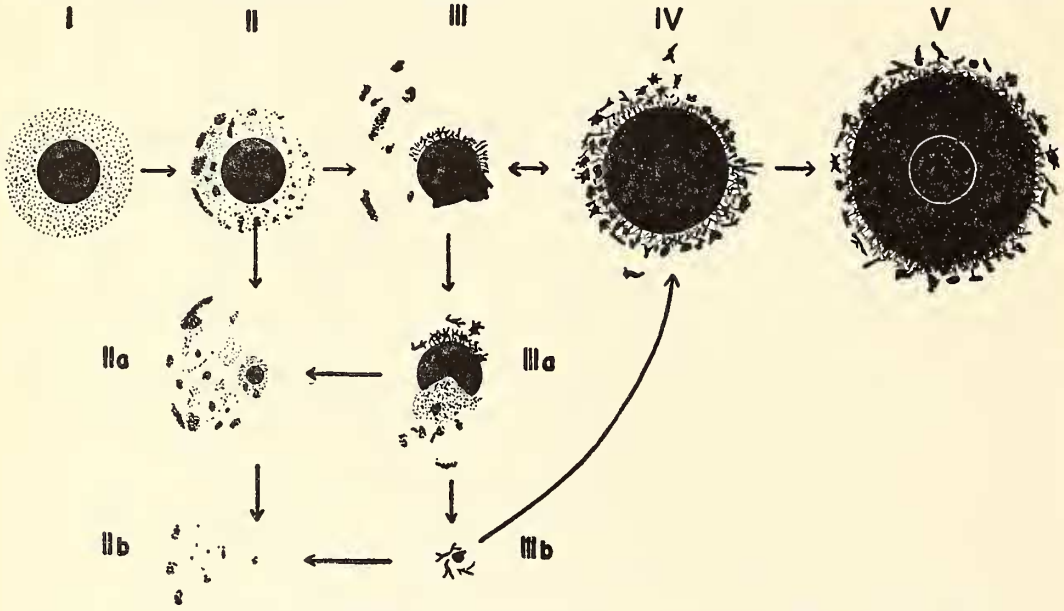



Text-fig. 3. Migration of melanocytes as traced by studying three melanocytes at the margin of a growing homotransplant. Melanocyte *a* travelled 170 microns in 120 hrs.; then, it could no longer be followed. Melanocyte *b* had a considerable accumulation of pigment, both near its center and around its edges (although this is not shown); it migrated hardly at all and after 72 hours it transformed into a melanophore; after this, it remained stationary. Melanocyte *c* migrated about 75 microns during the first 48 hours; during this time pigment developed at its center and at its periphery; then, at 72 hours, the cell stopped migrating and transformed into a melanophore. (Camera lucida, about 50×.)

was obtained in the present series when melanoma particles from hybrids were placed between the scales of the swordtail. This may possibly be explained by the fact that in the present heterotransplants swordtails of a highly inbred strain were used, whereas previously they were not. Text-fig. 4 shows a schematic representation of the results obtained in the present study.

Goodrich & Nichols (1933) transplanted, into unpigmented areas of the skin of goldfish, scales with their adhering dermal tissues containing some melanophores obtained from pigmented regions. They found that the melanophores persisted and invaded the surrounding unpigmented dermis. They also found that autotransplants of scales and their adhering tissues produced no tissue antagonism while homotransplants of similar elements did.

More than twenty years ago Gordon (1931) found that macromelanophores had to be present in platyfish × swordtail hybrids before a melanoma would develop spontaneously. It was also known from histological studies by Reed & Gordon (1931) that the definitive melanoma of these fish contained every conceivable gradation of cells between the darker, larger melanophores




TEXT-FIG. 4. Schematic representation of the observations on the various fates of melanoma implants.

I. Soon after transplantation the implant is surrounded by a gray halo of free melanin particles.

II. Macrophages (or, melanophages) engulf and remove the melanin detritus.

IIa. Some implants, particularly the heterotransplants, degenerate within a short time, breaking up and forming masses of melanin debris.

IIb. Last stage of implant degeneration, in which most of the melanin debris has been eliminated by the action of melanophages.

III. Dendritic processes of pigment cells appear at the margin of the implant; most of the melanin detritus is removed.

IIIa. Some implants after reaching this stage of growth begin to degenerate.

IIIb. Most of the implants that fall into the category indicated by IIIa degenerate completely. One implant, however, persisted at this stage for several months and then regenerated to grow to the next stage, IV.

IV. The implant is surrounded by many dendritic processes and by whole pigment cells. This is a critical period in the growth of the implant because regression may still take place up to about six weeks after implantation.

V. The growing transplant, in which the white circle represents the size of the original implant. Around the periphery of the growth free melanophores and melanocytes may be identified. These are the primary cells of the melanoma. Histological study of such transplants reveals the presence of a melanosis, the precursor of melanoma development, rather than a definitive melanoma.

and the lighter, smaller, irregular (stellate) pigment-containing cells which are now known to be melanocytes. These authors suggested that the smaller cells are the precursors of the larger ones. There has been no direct evidence of this important cell relationship up to the time that observations were made on the ontogeny of pigmented cells in the transplanted melanomas, as indicated in this paper. These observations were confirmed almost simultaneously by the study of regeneration of melanomatous dorsal fins of platyfish × swordtail hybrids by Ermin & Gordon (1952, 1954). The evidence from both sources is still fragmentary, but the new techniques of transplantation and regeneration should in future work add to our knowledge of the relationships, that is, the histogenesis and ontogeny, of these vitally important cells of the melanoma.

## Summary and Conclusions

1. Melanomas develop on the caudal peduncles and caudal fins of swordtail (*Xiphophorus montezumae* × *X. helleri*) hybrid fishes carrying the dominant macromelanophore gene for spotted caudal, *Sc*. Autotransplants of the melanomas have been successful. Homotransplants of the melanoma have also been successful in hosts having the dominant *Sc* gene. Transplants into other hosts either failed completely or were maintained only for short periods.

2. Transplantation of the tumor fragment into the corium under the scales and epidermis permits the continuous observation of individual cells in the growing implant, owing to the transparency of the thin tissues.

3. The histological characteristics of the implanted melanoma tissue (high vascularity, blood filled lacunae, lightly pigmented melanocytes) are not present in the well-established implant. After 24 months of activity, the original melanoma implant takes on the morphological features of a melanosis. Melanosis, a premelanomatous stage, is produced by the hypertrophic growth of macromelanophores.

4. Two types of immature pigment cells, or melanocytes, emerge from the implant. The smaller one (*small melanocyte*) is thin and has few and tenuous pseudopodial processes; the larger one (*large melanocyte*) is broad and has wide, blunt pseudopodia.

5. Some large melanocytes transform into macromelanophores.

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## EXPLANATION OF THE PLATES

### Plate I

Fig. 1. *montezumae* × *helleri* swordtail hybrids of stock h50*Sc*, with large, spontaneously developed *Sc* melanomas on their caudal peduncles and tails. In these lateral views the full dimensions of the melanomas cannot be appreciated, but actually each tumor was approximately a quarter of an inch thick. Note, in the male above, the rough dorsal and ventral surfaces of the caudal peduncle, and in the female below, the thickened lower lobe of the tail. Note the absence of black markings in the anterior areas of the hybrids.

(Photographs of fishes all life size).

Fig. 2. Homotransplant in a female *montezumae* × *helleri* h42*Sc* hybrid, above, and an autotransplant in a male hybrid, below. In the female the implant, obtained from its sibling, was placed in the belly region. The black areas in the tail developed spontaneously in response to the action of the *Sc* gene in the host. In the male, the implant, obtained from its own caudal fin melanoma, was placed in the middle area of the left side of the body. The black marking in the dorsal fin and the melanoma of the tail developed spontaneously in response to the *Sc* gene.

### Plate II

Fig. 1. Melanoma transplants in two male *montezumae* × *helleri* swordtail hybrids of strain h42*Sc*. In the male, above, the tumor tissue was removed from the melanoma in its tail and transplanted to its body just anterior and ventral to its dorsal fin. The hybrid was photographed 7 months after the implantation. In the male, below, the implant, obtained from its sibling, was placed just dorsal to the ventral fins and posterior to the pectoral fin. The other markings on the two fish are those spontaneously produced by macromelanophores in response to the *Sc* gene.

Fig. 2. An autotransplant and a heterotransplant of the spotted-dorsal melanoma originally developed spontaneously in response to the *Sd* gene in *helleri* × *maculatus* platyfish-swordtail hybrids. Above, an implant taken from the melanoma of the dorsal fin of a platyfish × swordtail hybrid was placed in the ventral region of the belly, just anterior to the ventral fins. The other melanotic areas developed spontaneously in response to the *Sd* gene. Below left, a melanoma taken from a platyfish × swordtail hybrid was placed by means of a trocar into the intermuscular region below the dorsal fin of an albino swordtail. To the right, the same albino swordtail showing a stage in the elimination (through the surface by means of macrophages) of most of the melanoma tissue. Both the heterotransplant and autotransplant were partially successful. (From a composite unpublished photograph of specimens studied by Gordon.)

### Plate III

History of a melanoma transplant in a *montezumae* × *helleri* swordtail hybrid of strain h42*Sc*. Photographs made from the living fish shown in Plate I, Fig. 2. Magnification 48×.

Fig. 1. Transplant, 0.8 × 0.7 mm, photographed within 24 hours after insertion. Fine melanin particles surround the implant.

Fig. 2. Within 36 hours, melanoma cells have grown out from the transplant; this may be seen best at the ventral surface. Macrophages (forming 3 large black masses) with engulfed free pigment particles appear to the right, along the edge of the striated scale. Note the group of macrophages just to the right of the implant and follow it in Figs. 3 and 4.

Fig. 3. Within 48 hours, growth of melanoma cells may be seen at the ventral region where focus is better, but growth extends from the dorsal region of the transplant as well. The pigment-laden macrophages

may be seen lined up along the distal margin of a scale.

FIG. 4. Within 72 hours, the melanomatous mass has spread. The pseudopodial or dendritic processes of the pigment cells extend beyond the main mass; those at the ventral margin are filamentous, those dorsally are broader.

FIG. 5. After 8 days, many pigment cells have wandered out of the main mass. The free pigment has been removed by macrophages; note that only two of the three larger groups of macrophages remain and that they are considerably smaller.

FIG. 6. Melanoma transplant in a *montezumae* × *helleri* hybrid, h42*Sc*, photographed two weeks after implantation. To the left, a vigorous outgrowth of pigment cells consisting of melanocytes and macromelanophores may be seen. The entire implant is surrounded by many micromelanophores which are the normal pigment cells of the host.

PLATE IV

Figs. 1 to 4. Photomicrographs of pigment cells growing along the margin of living melanoma implants.

FIG. 1. Small melanocyte, characterized by its long, filamentous dendritic processes and relatively small cell body, seen to the left.

FIG. 2. Large melanocytes in a stage of transformation to melanophores. Note the concentrations of pigment granules (1) at the peripheries of the broad branches, and (2) at the center of the cell, particularly around the nucleus of the lowermost cell.

FIG. 3. Large melanocytes and a macromelanophore. Two melanocytes are shown along the upper part of the photomicrograph, another below and to the left. To the bottom right, partly overlain by the melanocyte, a large melanophore may be seen.

FIG. 4. Melanophores derived from melanocytes —the one to the left still retains some features of the melanocyte with its broad branching processes.

FIG. 5. Cross-section through epidermis and corial tissues between the scales of a transplanted melanoma fixed after 8 months of growth. The epidermis (top) is slightly hyperplastic and has a heavily pigmented layer between it and the scale. Between the two scales the corial tissues are completely replaced by macromelanophores.

FIG. 6. Cross-section through the skin and underlying muscles of a melanoma transplant fixed after 8 months of growth. In addition to details illustrated in Fig. 5, this photomicrograph shows the subcutaneous invasion of the muscles by pigmented cells derived from the transplanted melanoma. The direct path of pigment cell invasion may be seen to the right. This type of growth has a parallel in the manner in which spontaneous melanomas develop in platyfish-swordtail hybrids described and illustrated by Gordon & Smith (1938).

FIG. 7. Whole mount of the ventral surface of a scale with its adhering pigmented tissues. The scale was removed from a fish that had an actively growing melanoma transplant for one week. The dorsal edge represents the distal margin of the scale. Here a few macrophage groups with their contained melanin particles are passing out through the epidermis. Other pigment-containing macrophages and a few dentritic melanocytes may be seen in the relatively clear epidermal area. The lower two-thirds of the photomicrograph reveal the pigment cells that have grown out from the transplant into the dermal tissue. The dendritic cells are mainly macromelanophores and melanocytes, some of which have disintegrated. The roundish masses of pigment represent melanophages (macrophages).

PLATE V

FIG. 1. Section through an *Sc* melanoma in a swordtail hybrid. (Photomicrograph by Dr. Ross F. Nigrelli).